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Title: Mice lacking the serotonin *Htr_{2B}* receptor gene present an antipsychotic-sensitive schizophrenic-like phenotype

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Running title: Schizophrenic-like phenotype of *Htr_{2B}*^{-/-} mice

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ABSTRACT

Impulsivity and hyperactivity share common ground with numerous mental disorders, including schizophrenia. Recently, a population-specific serotonin 2B (5-HT_{2B}) receptor stop-codon (i.e. *HTR2B* Q20*) was reported to segregate with severely impulsive individuals, while 5-HT_{2B} mutant (*Htr_{2B}^{-/-}*) mice also showed high impulsivity. Interestingly, in the same cohort, early-onset schizophrenia was more prevalent in *HTR2B* Q*20 carriers. However, the putative role of 5-HT_{2B} receptor in the neurobiology of schizophrenia has never been investigated. We assessed the effects of the genetic and the pharmacological ablation of 5-HT_{2B} receptors in mice subjected to a comprehensive series of behavioral tests screening for schizophrenic-like symptoms and investigated relevant dopaminergic and glutamatergic neurochemical alterations in the cortex and the striatum. Domains related to the positive, negative and cognitive symptom-clusters of schizophrenia were affected in *Htr_{2B}^{-/-}* mice, as shown by deficits in sensorimotor gating, in selective attention, in social interactions and in learning and memory processes. In addition, *Htr_{2B}^{-/-}* mice presented with enhanced locomotor response to the psychostimulants dizocilpine and amphetamine, and with robust alterations in sleep architecture. Moreover, ablation of 5-HT_{2B} receptors induced a region-selective decrease of dopamine and glutamate concentrations in the dorsal striatum. Importantly, selected schizophrenic-like phenotypes and endophenotypes were rescued by chronic haloperidol treatment. We report herein that 5-HT_{2B} receptor deficiency confers a wide spectrum of antipsychotic-sensitive schizophrenic-like behavioral and psychopharmacological phenotypes in mice and provide first evidence for a role of 5-HT_{2B} in the neurobiology of psychotic disorders.

INTRODUCTION

Schizophrenia is a devastating, complex and costly neuropsychiatric disorder that affects about 1% of the world's population (Olesen *et al*, 2012). Schizophrenia is typically characterized by three symptom clusters: positive (e.g. hallucinations, delusions, disordered thoughts), negative (e.g. flattened affects, social withdrawal) and cognitive (e.g. attention and working memory deficits) (Simpson *et al*, 2010). This disease has traditionally been associated with a deregulated dopaminergic system, mainly due to the fact that for many years typical antipsychotics (i.e. strong dopamine (DA)-D₂ receptor antagonists, such as haloperidol) have served as the mainstream pharmacotherapy for psychotic patients (Keshavan *et al*, 2008). However, it has long been known that the serotonergic system is also implicated in the pathogenesis of schizophrenia (Meltzer and Massey, 2011). Indeed, atypical antipsychotics, of which clozapine is the prototype, consist of agents that are more potent antagonists of serotonin 2A (5-HT_{2A}) receptors over D₂ receptors (Gonzalez-Maeso *et al*, 2008). Many atypical antipsychotics show similar affinity for 5-HT_{2B} and 5-HT_{2A} receptors (e.g. clozapine, amisulpride, asenapine, aripiprazole or cariprazine) (Abbas *et al*, 2009; Kiss *et al*, 2010; Shahid *et al*, 2009; Shapiro *et al*, 2003), although the contribution of this 5-HT_{2B} receptor to the action of antipsychotic compounds has never been reported. Notably, the elucidation of the intricate mechanisms underlying the neurobiology of schizophrenia is imperative in order to develop novel drugs with improved therapeutic efficacy (Kvajo *et al*, 2012). In this direction, models of genetic deletion in mice have been indispensable in characterizing the contribution of specific genes to disease pathophysiology (Arguello and Gogos, 2006).

Impulsivity, very broadly defined as action without foresight, novelty-seeking and hyperlocomotion share common ground with numerous mental disorders, including chronic substance abuse, attention deficit hyperactivity disorder and schizophrenia (Fineberg *et al*, 2010; Humby and Wilkinson, 2011). In a recent study conducted in a Finish cohort, we reported that a population-specific 5-HT_{2B} receptor stop-codon (*HTR2B* Q20*) segregates with severe impulsivity, psychosis and early-onset schizophrenia (Bevilacqua *et al*, 2010). Moreover, mice in which the *Htr_{2B}* gene has been genetically inactivated (i.e. *Htr_{2B}^{-/-}*) exhibit a hyperlocomotor phenotype as well as impulsivity, as assessed in the delay discounting task (Bevilacqua *et al*, 2010; Doly *et al*, 2008). However, the extent to which 5-HT_{2B} receptor is implicated in the neurobiology of schizophrenia has never been investigated.

In the present study, we assessed the effects of the genetic ablation of *Htr_{2B}* in mice subjected to a comprehensive series of behavioral tests screening for positive, negative and cognitive schizophrenic-like symptoms. Following the demonstration that loss of function of the 5-HT_{2B} receptor in *Htr_{2B}^{-/-}* mice confers a wide spectrum of schizophrenic-like behavioral phenotypes, we show that selected phenotypes are induced by acute pharmacological blockade of 5-HT_{2B} receptors with RS127445 (selective 5-HT_{2B} receptor antagonist) and rescued by chronic antipsychotic treatment with the typical antipsychotic drug haloperidol. The dorsal striatum (dSTR) and its connections with the prefrontal cortex (PFC) have been implicated in the pathogenesis of both the positive and the cognitive symptoms of schizophrenia (Howes *et al*, 2009; Simpson *et al*, 2010). Indeed, neurochemical alterations in the dopaminergic and glutamatergic corticostriatal circuits underlie a substantial portion of the dysfunction seen in schizophrenic patients (Gordon, 2010; Howes *et al*, 2009; Keshavan *et al*, 2008; Simpson *et al*, 2010). Thus, we further investigated relevant dopaminergic and glutamatergic neurochemical alterations in the PFC and the dSTR of *Htr_{2B}^{-/-}* mice. Altogether, our findings present the first evidence for a role of 5-HT_{2B} receptors in the neurobiology of schizophrenia.

MATERIALS AND METHODS

Animals

Male *Htr_{2B}^{-/-}* mice used in these experiments were in a 129S2/SvPas (129S2) background and wild type (*Htr_{2B}^{+/+}*) 129S2 mice (8-12 weeks old) used as controls were bred in our animal facility under standard 12 light/dark schedule, and housed in groups of 3-5 of the same genetic background and sex after weaning (Diaz *et al*, 2012). Animal experiments were conducted in accordance with standard ethical guidelines (National Institutes of Health's "Guide for the Care and Use of Laboratory animals", and European Directive 2010/63/UE) and were approved by the Ethics Committee for Animal Experiments (N° 1170.01).

Drug treatments

The selective 5-HT_{2B} receptor antagonist RS127445 (RS; 0.5 mg/kg; Tocris, France) was administered acutely (i.e. 1 h before testing) and intraperitoneally (i.p.), as in previous studies from our group (Diaz *et al*, 2012; Doly *et al*, 2008). The glutamatergic psychostimulant and N-methyl-D-aspartic acid (NMDA) receptor antagonist dizocilpine (MK-801; 0.5 mg/kg; i.p.; Sigma-Aldrich, France) and the dopaminergic psychostimulant amphetamine (10 mg/kg; i.p.; Sigma-Aldrich, France) were dissolved in saline and administered at a rate 10 mL/kg. Different cohorts of *Htr_{2B}^{+/+}* and *Htr_{2B}^{-/-}* mice were chronically treated (4 weeks) with haloperidol (Sigma-Aldrich, France) in drinking water (2.0 mg/kg/day) or vehicle, as described before (Terry *et al*, 2007). Following chronic antipsychotic treatment, locomotor activity and prepulse inhibition (PPI) were tested, as described below. Separate mouse cohorts were used for the assessment of sleep architecture and for social interaction behavior and neurochemical estimations.

Locomotor response to novelty and to psychostimulants

Hyperactivity in response to novelty and locomotor hypersensitivity to acute administration of psychostimulants are considered useful correlates of positive schizophrenic-like symptoms in mice (Arguello *et al*, 2006). Locomotor activity was measured in a circular corridor with four infrared beams placed at every 90° (Imetronic, Passac, France), as previously described (Blundell *et al*, 2010; Doly *et al*, 2008). Mice were injected with saline and individually placed in the activity box for 2 h during four consecutive days in order to habituate to the apparatus. On the fifth day mice were placed in the activity box for 1 h and then treated with, dizocilpine or amphetamine. Locomotor activity was then screened for 2-6 h.

Prepulse Inhibition (PPI) of acoustic startle

PPI has been considered an operational measure of sensorimotor gating and pre-attentive processing, and its deficit has been consistently reported in schizophrenic patients and in relevant animal models (Meyer *et al*, 2005). Sensorimotor gating was assessed as previously described (Yadav *et al*, 2011). PPI was indexed by the percentage inhibition of the startle response at each level of prepulse intensity by using the following formula: % PPI = [(mean reactivity on pulse-alone trials- mean reactivity on prepulse-pulse trials)/mean reactivity on pulse-alone trials] x 100%. % Average PPI values were calculated from all PPI values across all the four prepulse intensities.

Sociability and preference for social novelty

Decreased interactions with conspecifics are often used to model social withdrawal, a negative symptom of schizophrenia (Arguello *et al*, 2006). The three-chamber social test was performed as previously described (Peca *et al*, 2011). Target subjects (Social I and Social II) were adult *Htr_{2B}^{+/+}* males. For the sociability test, the test animal was introduced into the middle chamber and was left to habituate for 5 min, after what an unfamiliar mouse (Social I) was introduced

into a wire cage in one of the side-chambers and an empty wire cage (EC) on the other side-chamber. The dividers were then opened and the test animal was allowed to freely explore all three chambers over a 10 min session. Preference for social novelty was assayed during a second 10 min session in which a novel stranger mouse (Social II) was inserted into the previously empty wire cage. The time spent in close interaction with the two cages was scored manually.

Novel Object Recognition (NOR)

Cognitive deficits, including working memory impairment, are typically observed in animal models of schizophrenia (Arguello *et al*, 2006; Meyer *et al*, 2005). The NOR task represents a most useful method for studying cognitive impairment in schizophrenia (Lyon *et al*, 2012). The NOR test was performed as previously described (Carlini *et al*, 2008) with retention tests performed at 1 h and at 24 h intervals in order to assess short- and long-term memory, respectively. Each trial lasted for 5 min. The time that the animals spent exploring the novel and the familiar objects were measured; results are expressed as % time of exploration of the novel object.

Contextual and cued fear conditioning

Cognitive deficits in associative fear memory have been reported in mice displaying schizophrenic-like phenotypes (Arguello *et al*, 2006; Gleason *et al*, 2012). Fear conditioning was assessed in computer-controlled operant chambers (Imetronic, Pessac, France), as previously described (Blundell *et al*, 2010; Cai *et al*, 2006; Powell *et al*, 2004). All results were expressed as % time freezing.

Latent Inhibition (LI)

Latent inhibition (LI) refers to the retardation in learning about the significance of a stimulus as a result of its prior repeated pre-exposures without consequence (Lubow and Moore, 1959). LI was assessed in a conditioned freezing paradigm, as previously described (Meyer *et al*, 2005; Willi *et al*, 2010). The test procedures consisted of four phases: pre-exposure, conditioning, context test, and tone test. Animals were randomly allocated to either the pre-exposed (PE) or non-pre-exposed (NPE) condition. The establishment of LI (i.e. difference in freezing between NPE and PE groups) reflects the ability to ignore stimuli that historically predict non-significant consequences. Thus, LI deficiency is a translational model that assesses attentional deficits in animal models of schizophrenia (Arguello *et al*, 2006; Meyer *et al*, 2005).

Sleep/wakefulness patterns

A battery of evidence shows that schizophrenia is associated with alterations in sleep architecture (Keshavan *et al*, 2008; Monti and Monti, 2004). Sleep architecture and assessment and spectral analysis of the electroencephalogram (EEG) recording were performed as previously described (Boutrel *et al*, 1999). Polygraphic recordings were scored visually every 15 s epoch as wakefulness, non-rapid eye movement (NREM) sleep, or rapid eye movement (REM) sleep, following classical criteria (Lena *et al*, 2004; Tobler *et al*, 1997), using Somnologica®. For analysis of the spontaneous sleep–wakefulness patterns, the amounts of vigilance states for each animal were calculated for every hour throughout 48 h and summed over 24 h. REM sleep latency was calculated as the time elapsing from sleep onset after the animal had been awakened, to the first episode of REM sleep (Popa *et al*, 2005).

Neurochemical Assessments

Dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and glutamate tissue concentrations in the PFC and the dSTR of *Htr_{2B}^{+/+}* and *Htr_{2B}^{-/-}* mice were assessed *ex vivo* by means of high-performance liquid chromatography (HPLC) as previously described (Banas *et*

al, 2011; Pitychoutis *et al*, 2011). A real-time qPCR-based approach was implemented to examine the mRNA expression of relevant key-receptor genes pertaining to the dopaminergic and the glutamatergic neurochemical systems. qPCR was performed as previously described (Diaz *et al*, 2012).

Statistical Analysis

Differences between experimental groups, were analyzed by unpaired t-tests, one-way or two-way analysis of variance (ANOVA) with genotype and treatment as main factors, depending on the experimental design. Bonferroni's *post-hoc* test was applied in order to elucidate specific differences between groups. Repeated measures ANOVAs were implemented in order to analyze locomotor activity and sleep architecture data. Statistical Analyses of the results are summarized in Supplementary Tables (**Table S1-S2**). In graphs/tables the values represent means \pm SEM and $p < 0.05$ was considered statistically significant.

RESULTS

Deficits in the PPI of the startle reflex

Genetic ablation of the 5-HT_{2B} receptor induced a global deficit in the PPI of the startle reflex (**Fig. 1a**) and a reduction in startle amplitude (**Fig. S1a**). Moreover, acute treatment of *Htr_{2B}^{+/+}* mice with the 5-HT_{2B} receptor antagonist RS127445, phenocopied the effects of genetic ablation of 5-HT_{2B} receptor in the PPI (**Fig. 1b**; **Fig. S1b**)

Enhanced locomotor response to psychostimulants

Administration of NMDA receptor antagonists (e.g. dizocilpine) and DA releasers (e.g. amphetamine) exacerbate existing symptoms in schizophrenic patients and cause psychotic-like symptoms in humans and in rodents (Arguello *et al*, 2006). As shown herein and before, *Htr_{2B}^{-/-}* mice exhibit enhanced locomotor response to novelty (**Fig. 1c**) (Doly *et al*, 2008). The locomotor response of *Htr_{2B}^{-/-}* mice to both dizocilpine (**Fig. 1d**) and amphetamine (**Fig. 1e**) was significantly enhanced, as compared to *Htr_{2B}^{+/+}* mice. Several other behaviors appear to be completely unaltered in *Htr_{2B}^{-/-}* mice, including motor coordination on the Rotarod and nociception assessed in the hot-plate (**Fig. S1c,d**).

Defect in social interaction

According to our data, both *Htr_{2B}^{-/-}* mice and RS-treated *Htr_{2B}^{+/+}* mice displayed dysfunctional social interaction behavior (**Fig. 1f**), as measured by observing the time mice spent in close interaction with the social partner *vs.* the inanimate stimulus (i.e. empty wire cage; EC). During the second session, preference for social novelty was evaluated by inserting a novel social stimulus (social II) in the previously empty cage and measuring the time spent interacting with Social I and Social II stimuli (**Fig. 1g**). All three groups showed preference for social novelty as revealed by the greater % time interacting with Social II *vs.* Social I (**Fig. S1e**). Social memory was assessed by comparing the time mice spent in close interaction with Social I stimulus during the two different trials. This analysis showed that genetic ablation of 5-HT_{2B} receptor impaired social memory, since *Htr_{2B}^{-/-}* mice spent similar time interacting with Social I partner during both trials (**Fig. 1h**). Social memory in both *Htr_{2B}^{+/+}* and *Htr_{2B}^{+/+}*-RS- treated mice appeared to be intact. In addition, social interaction with a juvenile conspecific was also impaired in *Htr_{2B}^{-/-}* mice (**Fig. S1f**). Of note, the differences observed in social interaction could not be attributed to altered olfactory function due to the genetic ablation of 5-HT_{2B} receptor in mice (**Fig. S1g,h**).

Memory deficits in the NOR and the fear conditioning

5-HT_{2B} receptor deficiency induced a significant impairment of both short- (1 h; **Fig. 2a**) and long-term NOR memory (24 h; **Fig. 2b**). Similarly, *Htr_{2B}^{-/-}* mice exhibited impaired fear learning and memory, as evidenced by the reduced time they spent freezing in response to the

context (**Fig. 2c**) and the cue in a fear conditioning paradigm (**Fig. 2d**). These cognitive deficits appear to be independent of object exploration behavior or sensitivity to foot shocks. Indeed, *Htr_{2B}^{-/-}* and *Htr_{2B}^{+/+}* mice spent similar time exploring the objects during both NOR trials (**Fig. S1i**) and showed comparable freezing behavior in response to foot shock during training in the fear conditioning paradigm (**Fig. S1j**).

Attention deficits in the LI paradigm

In the test of the conditioned response to the tone, the presence of LI in *Htr_{2B}^{+/+}* mice was demonstrated by a clear reduction in the amount of freezing to the tone in pre-exposed (PE) mice relative to the non-pre-exposed (NPE) mice. However, LI was not established in *Htr_{2B}^{-/-}* mice (**Fig. 2e**).

Alterations in sleep architecture

Htr_{2B}^{-/-} mice exhibited increased wakefulness duration at the expense of NREM sleep (**Fig. 3a**), as well as decreased latency to REM sleep (**Fig. 3b**). Under baseline conditions, *Htr_{2B}^{+/+}* and *Htr_{2B}^{-/-}* mice exhibited the typical polyphasic structure of vigilance states found in rodents and a diurnal rhythm of sleep and wakefulness, with larger amounts of sleep during the light period classically observed in nocturnal species (**Fig. 3c-e**). However, *Htr_{2B}^{-/-}* mice exhibited significantly increased amounts of wakefulness, at the expense of NREM sleep, throughout the dark period and at the onset of light period (**Fig. 3c,d**) while REM sleep was not affected (**Fig. 3e**).

Chronic haloperidol treatment rescued selected schizophrenic-like phenotypes in *Htr_{2B}^{-/-}* mice

Haloperidol was selected herein on the basis of its minor binding capacity to 5-HT₂ receptors including murine 5-HT_{2B} receptors unlike many other antipsychotics (Meltzer, 2013) (**Fig. S1k**). Chronic oral intake of haloperidol (2mg/kg/day) for 4 weeks restored the balance between wakefulness and NREM sleep (**Fig. 3c-e**) in *Htr_{2B}^{-/-}* mice. Moreover, chronic haloperidol treatment normalized the PPI deficits (**Fig. 3f**) and the psychomotor agitation (**Fig. 3g**) conferred by 5-HT_{2B} receptor deletion. However, haloperidol treatment did not ameliorate the social deficit observed in *Htr_{2B}^{-/-}* mice in the 3-chamber social interaction test (**Fig. 3h,i**).

Neurochemical alterations in *Htr_{2B}^{-/-}* mice

In our experimental setup, DA, and glutamate tissue levels were found decreased in the dSTR of *Htr_{2B}^{-/-}* mice, while in the PFC no major neurochemical alterations were observed (**Fig. 4a,b**). Moreover, our results showed that the genetic ablation of the 5-HT_{2B} receptor resulted in a differential regulation of mRNA expression of DA and glutamate receptors in the PFC and the dSTR. In particular in the dSTR, D₂R mRNA expression was decreased (**Fig. 4c**), while GluR2 was upregulated in the PFC (**Fig. S2f**). It is noteworthy, that chronic haloperidol treatment normalized the observed neurochemical alterations in DA and glutamate concentrations (**Fig. 4a,b**) and D₂R receptor expression in the dSTR (**Fig. 4c**).

DISCUSSION

Herein we investigated the effects of the genetic ablation of 5-HT_{2B} receptor across a battery of translational behavioral paradigms relevant for assessing face validity of animal models of schizophrenia (Arguello *et al.*, 2006; Meyer *et al.*, 2005; Willi *et al.*, 2010). We report herein that loss of function of 5-HT_{2B} receptor confers a wide spectrum of schizophrenic-like behavioral and psychopharmacological phenotypes in mice. Importantly, domains related to the positive, negative and cognitive symptom-clusters of schizophrenia appear to be affected upon 5-HT_{2B} receptor gene ablation (**Table 1**). *Htr_{2B}^{-/-}* mice display a global deficit in sensorimotor gating (i.e. impaired PPI performance) as well as psychomotor agitation (i.e.

novelty-induced hyperlocomotion), psychostimulant hypersensitivity (i.e. enhanced locomotor response to psychostimulants), all phenotypes that have been related to the positive symptoms of schizophrenia (Gunduz-Bruce, 2009; Meyer *et al*, 2005; Pratt *et al*, 2012). Moreover, 5-HT_{2B} receptor gene ablation impaired social interaction behavior with conspecifics, a trait used commonly to model negative symptoms of schizophrenia in rodents (Arguello *et al*, 2006; Pratt *et al*, 2012). Most importantly, *Htr_{2B}^{-/-}* mice present with selective attention deficits (i.e. lack of LI establishment) and learning and memory impairments (i.e. poor NOR and fear conditioning performance and social memory deficit) that closely resemble the cognitive deficits observed in schizophrenic patients (Keshavan *et al*, 2008). Remarkably, the alterations in sleep architecture observed in *Htr_{2B}^{-/-}* mice (i.e. reduced duration of NREM sleep and decreased latency to REM sleep) mimic the sleep abnormalities often seen in schizophrenic patients (Keshavan *et al*, 2008). The list of behavioral paradigms implemented herein, although not exhaustive, is consistent with schizophrenia-like behavioral abnormalities. Moreover, selected schizophrenic-like phenotypes (i.e. PPI and social interaction deficits) were phenocopied in *Htr_{2B}^{+/+}* mice by acute pharmacological ablation of 5-HT_{2B} receptors with the selective receptor antagonist RS127445.

We next assessed whether selected schizophrenic-like phenotypes could be rescued by chronic antipsychotic treatment. For this reason *Htr_{2B}^{-/-}* and *Htr_{2B}^{+/+}* were chronically treated with the typical antipsychotic haloperidol. Of note, chronic antipsychotic treatment completely abolished the observed PPI deficits and alterations in sleep architecture. As expected, haloperidol treatment suppressed psychomotor agitation in *Htr_{2B}^{-/-}* mice, but was ineffective against social withdrawal, a negative symptom of schizophrenia. Moreover, chronic haloperidol treatment also had an effect in *Htr_{2B}^{+/+}* mice, normalizing the differences observed between the two genotypes during locomotor activity and social interaction testing.

How 5-HT_{2B} receptor gene deficiency may lead to the observed schizophrenic-like phenotype can only be speculated at present since the function of this receptor in the brain remains largely uninvestigated. It is noteworthy that 5-HT_{2B} receptors have been shown to modulate aspects of both serotonergic and dopaminergic neurotransmission (Bevilacqua *et al*, 2010; Diaz and Maroteaux, 2011; Doly *et al*, 2008). In particular, *ex vivo* studies have indicated that 5-HT_{2B} receptors may serve as positive autoregulator of the serotonergic tone through modulating the 5-HT transporter (SERT) in raphe neurons (Launay *et al*, 2006), while *in vivo* studies in mice further confirmed that 5-HT_{2B} receptors contribute to the behavioral effects of the SERT-targeting 5-HT releasers, MDMA (i.e. ecstasy) and dexfenfluramine (Banas *et al*, 2011; Doly *et al*, 2009; Doly *et al*, 2008). Recently, Auclair *et al*. reported that 5-HT_{2B} receptors control the mesoaccumbal DA pathway activity, as an acute systemic administration of the 5-HT_{2B} subtype-selective antagonist RS127445 in rats induced a region-selective reduction of extracellular DA levels in the NAC in response to amphetamine challenge (Auclair *et al*, 2010).

In light of the schizophrenic-like phenotype observed herein, the relevant neurochemical alterations found are not surprising. Indeed, the enhanced psychomotor response of *Htr_{2B}^{-/-}* mice to dizocilpine and to amphetamine indicates that the glutamatergic and/or the dopaminergic systems must, at least to some extent, be altered in *Htr_{2B}^{-/-}* mice. Schizophrenia has been largely associated with dopaminergic hypofunction in the PFC and hyperfunction in the basal ganglia; indeed numerous post-mortem studies report increased D₂R densities in the dSTR (Hirvonen *et al*, 2005; Seeman and Kapur, 2000) and decreased D₂R levels in the PFC of schizophrenic patients (Takahashi *et al*, 2006). Moreover, aberrations in glutamate-mediated neurotransmission through NMDA receptors in schizophrenia is supported by the reduced glutamate levels in the cerebrospinal fluid (CSF) and NMDA receptor expression in the PFC of schizophrenic patients (Gordon, 2010; Keshavan *et al*, 2008). In our experimental setup, DA and glutamate tissue concentrations and D₂R mRNA expression were found decreased in the dSTR of *Htr_{2B}^{-/-}* mice. In accordance with our findings, DA tissue concentrations were also found decreased in the dSTR of *Nogo-A^{-/-}* mice that also present with a schizophrenic-like phenotype (Willi *et al*, 2010). Intriguingly, the observed dopaminergic

and glutamatergic alterations in the dSTR were corrected upon chronic antipsychotic treatment. The latter finding indicates that both D₂R-related and unrelated defects in *Htr_{2B}^{-/-}* mice are reversed by haloperidol treatment and provide evidence that the effects of 5-HT_{2B} receptors extend beyond the serotonergic and the dopaminergic systems.

Acute pharmacological ablation of 5-HT_{2B} receptors (i.e. RS127445 treatment) phenocopied both positive (i.e. PPI deficit) and negative (i.e. impaired social interaction) symptoms observed in *Htr_{2B}^{-/-}* mice. Moreover, in an earlier study, administration of another 5-HT_{2B} receptor-selective antagonist (i.e. SB-215505) was shown to increase wakefulness at the expense of NREM and REM sleep in rats (Kantor *et al*, 2004) and in another study it was concluded that 5-HT exerts a 5-HT_{2B} receptor-mediated facilitation of NREM sleep (Popa *et al*, 2005), which are in accordance with our findings in *Htr_{2B}^{-/-}* mice. Thus, based on these observations, we propose that the schizophrenic-like phenotype of *Htr_{2B}^{-/-}* mice results from a combination of both the direct absence of 5-HT_{2B} receptor signaling and the neural adaptations triggered by the permanent lack of this receptor.

Antipsychotic drugs ameliorate hallucinations and delusions in patients with neuropsychiatric disorders, particularly schizophrenia and bipolar disorders. The two main classes are known as typical and atypical antipsychotics. Many atypical antipsychotics show similar affinity for 5-HT_{2B} and 5-HT_{2A} receptors (e.g. clozapine, amisulpride, asenapine) (Abbas *et al*, 2009; Shahid *et al*, 2009) or even higher affinity for 5-HT_{2B} receptors (e.g. aripiprazole, cariprazine) (Kiss *et al*, 2010; Shapiro *et al*, 2003). Notably, it was recently shown that the efficacy of clozapine, but not haloperidol, is diminished in *Pet1^{-/-}* mice, that lack 5-HT neurons, and thus depends on an intact presynaptic serotonergic system (Yadav *et al*, 2011). Given that many marketed atypical antipsychotic drugs present high affinity for 5-HT_{2B} receptors (Shapiro *et al*, 2003) and that these receptors are expressed in 5-HT neurons (Diaz *et al*, 2012), our findings bear broader significance for the elucidation of the effects of these drugs in the treatment of psychotic disorders.

It should be borne in mind that the behavioral phenotype of *Htr_{2B}^{-/-}* mice portrayed herein is not exclusive of a putative role of the 5-HT_{2B} receptor in the neurobiology of a broader range of neuropsychiatric diseases that share common domains of dysfunction with psychotic disorders. Interestingly, it was recently reported that two polymorphisms of the *HTR2B* gene were associated with intelligence quotiation, intellectual disability and language onset delay in a cohort of children and young adults suffering from autistic spectrum disorders (Hervas *et al*, 2014). In agreement, present data strongly support a novel role for 5-HT_{2B} in the regulation of cognitive function and social behavior. Despite the fact that *Htr_{2B}^{-/-}* mice display deficits in social interaction and in learning and memory processes that closely resemble autistic-like behavior, they also present with deficits in sensorimotor gating and locomotor hypersensitivity to psychostimulants which are core endophenotypes of schizophrenia (Arguello *et al*, 2006; Powell *et al*, 2009).

Overall, the present study revealed that 5-HT_{2B} receptor deficiency induces a wide spectrum of antipsychotic-sensitive schizophrenic-like phenotypes in mice. Most importantly, further pharmacological validations with selective 5-HT_{2B} receptor antagonists (given acutely or chronically) that extend present findings to other behavioral tests, mouse strains and species, or other brain regions and neurochemical systems are clearly warranted to further dissect the role of the 5-HT_{2B} receptor in the neurobiology of psychotic spectrum disorders. This genetic mouse model holds additional value in further elucidating the schizophrenia-relevant neurodevelopmental, epigenetic and physiological mechanisms that may be sensitive to 5-HT_{2B} receptor polymorphisms.

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FIGURE LEGENDS

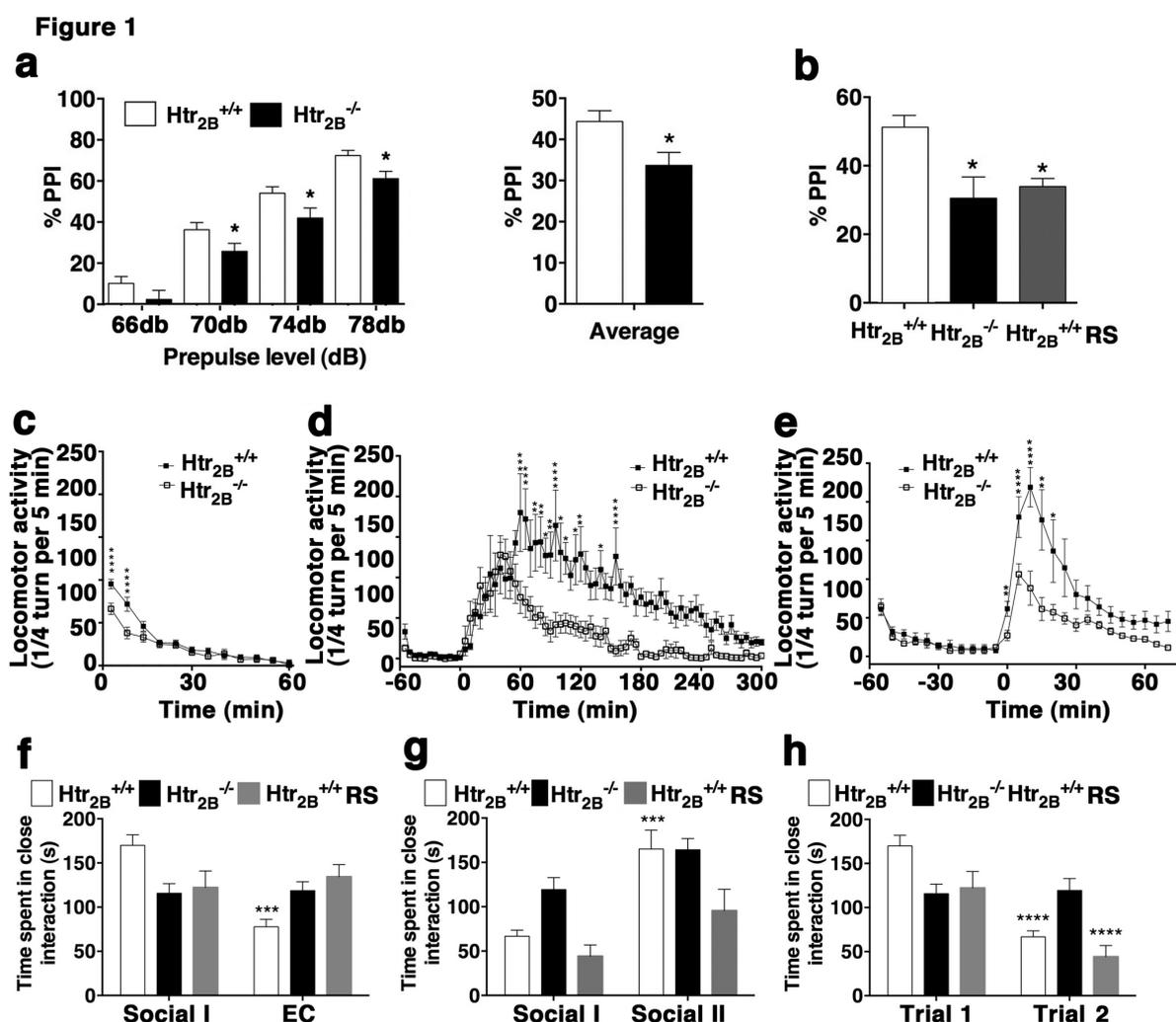


Figure 1: Prepulse inhibition (PPI) is impaired in $Htr_{2B}^{-/-}$ mice across prepulse intensities over 66db and in average of all intensities (N=27 per group) (a). Acute injection of RS127445 (0.5 mg/kg), a selective 5-HT_{2B} receptor antagonist (RS) also impaired PPI in $Htr_{2B}^{+/+}$ mice (N=8 per group) (b). Genetic ablation of the 5-HT_{2B} receptor enhanced novelty-induced locomotion during the first 10 min of testing (N=8 per group) (c). Genetic blockade of the 5-HT_{2B} receptor enhanced locomotor responses to the glutamatergic psychostimulant and non-competitive NMDA receptor antagonist dizocilpine (MK-801; 0.5 mg/kg; i.p.) (N=8 per group) (d) and to the dopaminergic psychostimulant amphetamine (10 mg/kg; i.p.) (N=8 per group) (e). Genetic and pharmacological ablation of the 5-HT_{2B} receptor impaired social interactions, as evidenced by the lack of preference for the social partner (social I vs. the empty wire cage; EC) (N=7-9 per group) (f). $Htr_{2B}^{+/+}$ mice spent significantly more time interacting with the novel social stimulus (Social II vs. Social I), but this was not the case for $Htr_{2B}^{-/-}$ mice or $Htr_{2B}^{+/+}$ -RS-treated mice (N=7-9 per group) (g). Genetic ablation of 5-HT_{2B} receptor impaired social memory, since $Htr_{2B}^{-/-}$ mice spent similar time interacting with Social I partner during both trials, whereas both $Htr_{2B}^{+/+}$ and $Htr_{2B}^{+/+}$ -RS-treated mice spent significantly less time interacting with Social I partner in the second trial (N=7-9 per group) (h). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ significantly different from vehicle-treated $Htr_{2B}^{+/+}$ mice as determined by unpaired t-test, one way ANOVA or 2-way ANOVA; full statistical analysis is presented in Table S1.

Figure 2

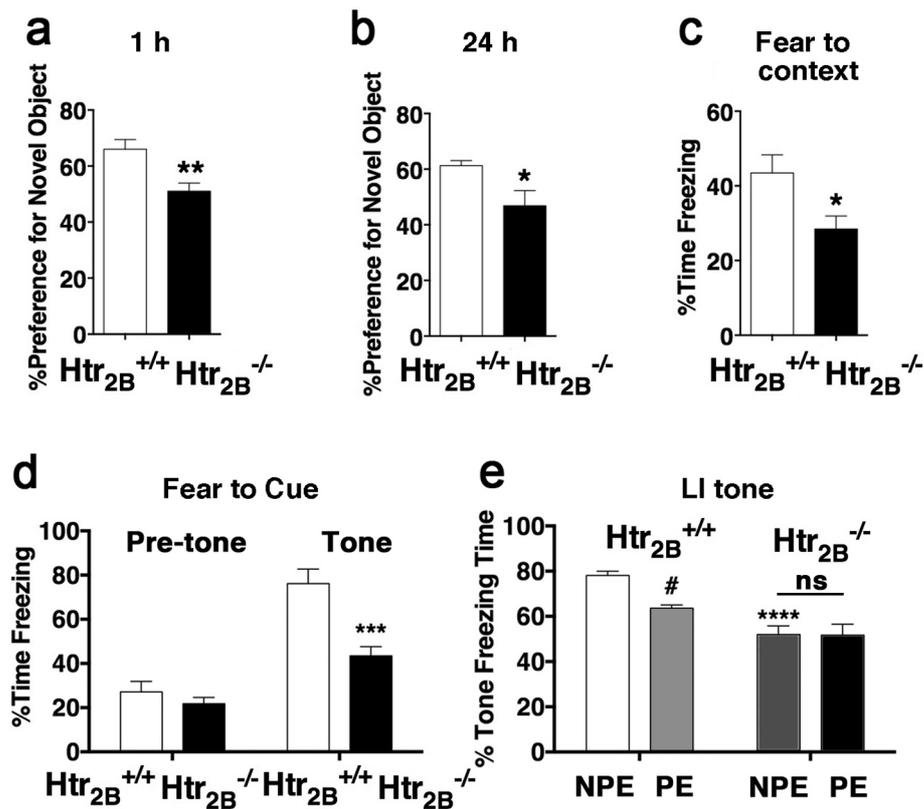


Figure 2: $Htr_{2B}^{-/-}$ mice present with impaired short- (a) and long-term (b) memory for a novel object, as assessed in the NOR test (N=8 per group). Contextual (c) and cued (d) fear memory is impaired in $Htr_{2B}^{-/-}$ mice (N=6-7 per group). In the latent inhibition (LI) paradigm, LI was established in $Htr_{2B}^{+/+}$ but not in $Htr_{2B}^{-/-}$ mice as indicated by the absence of difference in freezing between NPE and PE groups (N=7 per group) $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ significantly different from $Htr_{2B}^{+/+}$ mice following unpaired t-test or 2-way ANOVA; $\#p < 0.05$ significantly different from NPE in 2-way ANOVA; full statistical analysis is presented in **Table S1**.

Figure 3

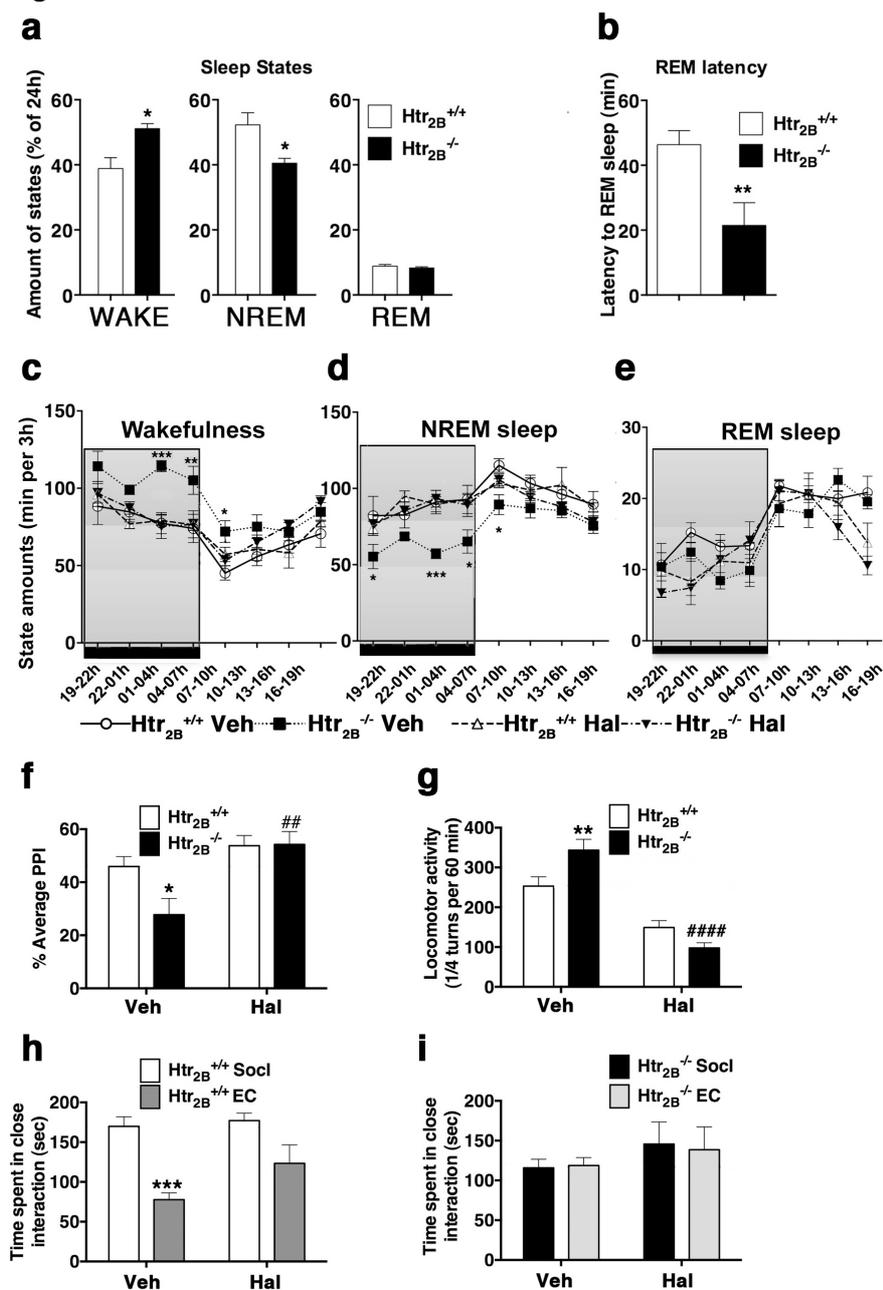


Figure 3: In $Htr_{2B}^{-/-}$ mice across 24 h, the total amount of wakefulness is increased while that of NREM sleep is decreased (**a**), as is the latency to REM sleep, calculated as the time elapsing from sleep onset after the animal had been awakened to the first episode of REM sleep (**b**) (N=4-5 per group). $Htr_{2B}^{-/-}$ mice exhibit significantly increased amounts of wakefulness at the expense of NREM sleep throughout the dark period and at the onset of the light period as assessed by the amount of vigilance states, expressed as minutes per 3 h (**c,d**) but REM sleep is not affected (**e**). Chronic oral haloperidol treatment (Hal, 2 mg/kg/day during 4 weeks) normalizes sleep-wakefulness states across the light/dark cycle (N=4-5 per group) (**c-e**). The effects of chronic oral haloperidol treatment were also tested in the prepulse inhibition (**f**) and locomotor activity (**g**) (N=8-10 per group); social interaction was assessed in the 3-chamber sociability test by comparing the time $Htr_{2B}^{+/+}$ (**h**) and $Htr_{2B}^{-/-}$ mice (**i**) spent interacting with a novel mouse (Soc I) vs. an empty wire cage (EC; N=7-9 per group). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ significantly different from the Veh-treated $Htr_{2B}^{+/+}$ mice; # $p < 0.05$; #### $p < 0.0001$ significantly different from the Veh-treated $Htr_{2B}^{-/-}$ mice; full statistical analysis is presented in **Table S1**.

Figure 5

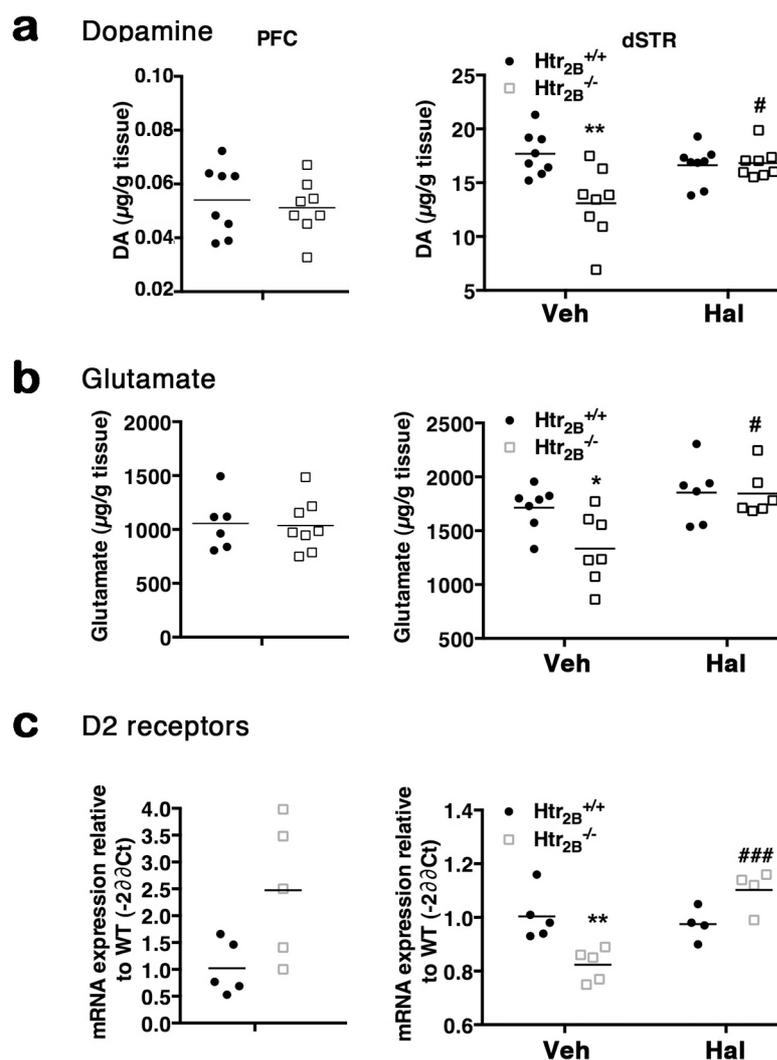


Figure 4: Region-distinctive neurochemical profile defects in the prefrontal cortex (**PFC-left**) and the dorsal striatum (**dSTR-right**) of $Htr_{2B}^{+/+}$ (black circle) and $Htr_{2B}^{-/-}$ (open square) mice. $Htr_{2B}^{-/-}$ mice present lower tissue concentrations of DA (**a**) and glutamate (**b**) in the dSTR (N=6-8 per group). Genetic ablation of the 5-HT_{2B} receptor was associated with a down-regulation of D₂R mRNA expression in dSTR (**c**) (N=4-5 per group). Neurochemical alterations in the dSTR are effectively reversed upon chronic haloperidol treatment (2 mg/kg/day during 4 weeks) (Hal) (**a-c**). * $p < 0.05$; ** $p < 0.01$; significantly different from Veh-treated $Htr_{2B}^{+/+}$ mice in unpaired t-test or 2-way ANOVA; # $p < 0.05$, ### $p < 0.001$ significant effect from Veh-treated $Htr_{2B}^{-/-}$ mice in 2-way ANOVA; full statistical analysis is presented in **Table S1**.

Table 1

Schizophrenia-like behavioral phenotypes in *Htr2B*^{-/-} mice

| Functional domain | Factor tested | <i>Htr2B</i> ^{-/-} | Reference |
|----------------------------|--|-----------------------------|-----------|
| Behavior | | | |
| Locomotor activity | Response to novelty | ↑ | Fig. 1c |
| | Response to dizocilpine | ↑ | Fig. 1d |
| | Response to amphetamine | ↑ | Fig. 1e |
| Sensorimotor gating | Prepulse inhibition | ↓ | Fig. 1a,b |
| Attention | Latent Inhibition | X | Fig. 2e |
| Cognitive function | Novel Object Recognition memory (short-term) | ↓ | Fig. 2a |
| | Novel Object Recognition memory (long-term) | ↓ | Fig. 2b |
| | Contextual fear memory | ↓ | Fig. 2c |
| | Cued fear memory | ↓ | Fig. 2d |
| | Social memory | ↓ | Fig. 1h |
| Social Interaction | Sociability | ↓ | Fig. 1f |
| | Juvenile conspecific | ↓ | Fig. S1f |
| Sleep architecture | Wakefulness | ↑ | Fig. 3a,c |
| | NREM sleep | ↓ | Fig. 3a,d |
| | REM sleep | - | Fig. 3a,e |
| | REM latency | ↓ | Fig. 3b |